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Zebra Mussel Research Program

Thermal Tolerance of Zebra Mussels (*Dreissena polymorpha*) Relative to Rate of Temperature Increase and Acclimation Temperature

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Prepared for Headquarters, U.S. Army Corps of Engineers

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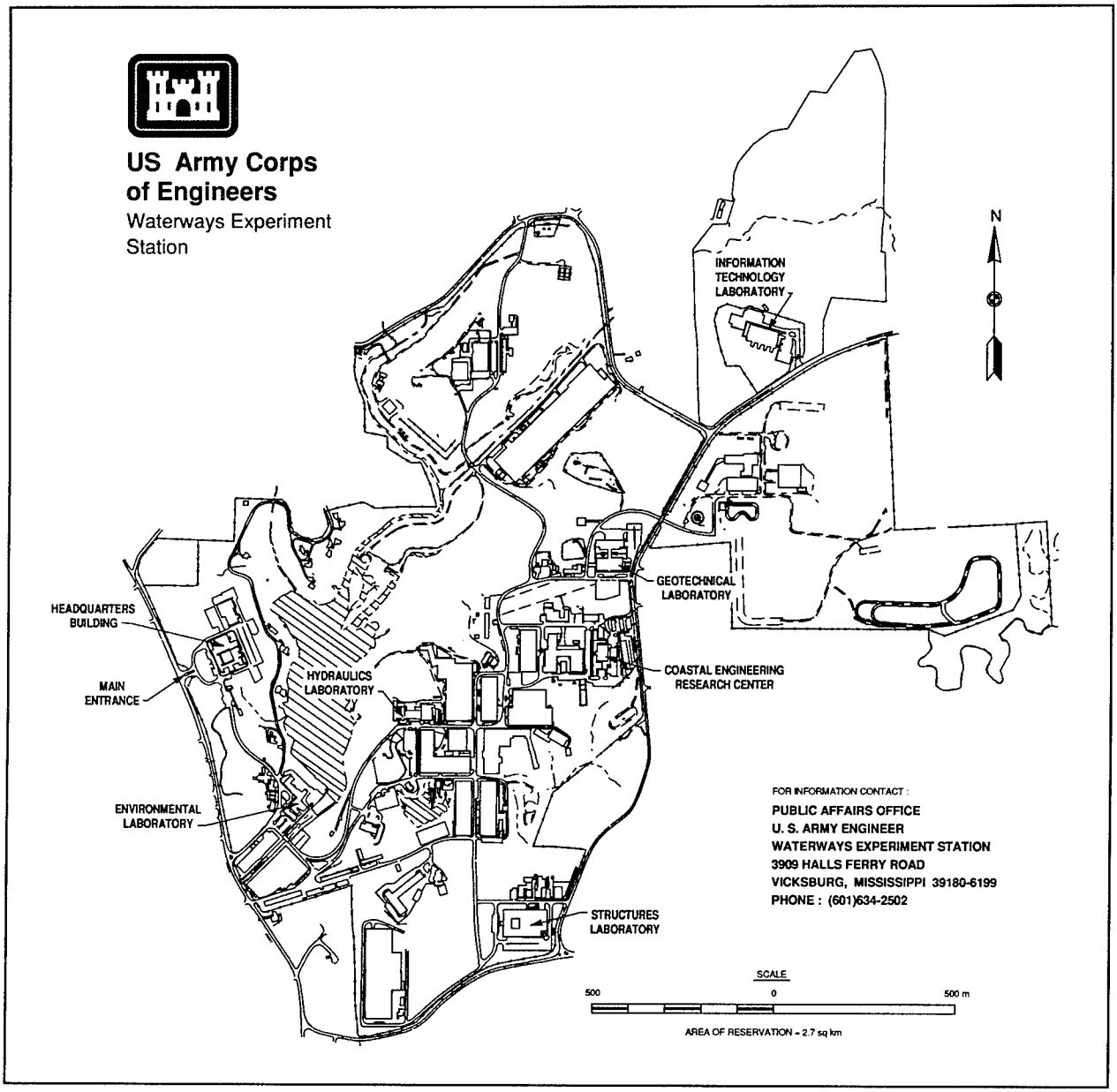

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Contents

Preface

The Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990 specified that the Assistant Secretary of the Army, Civil Works, will develop a program of research and technology development for the environmentally sound control of zebra mussels (*Dreissena polymorpha*). As a result, the U.S. Army Engineer Waterways Experiment Station (WES) initiated a program to develop control strategies for this species.

This report was prepared by Dr. Robert F. McMahon and Mr. Thomas A. Ussery, Center for Biological Macrofouling Research, University of Texas at Arlington, Arlington, TX. Ms. Diana M. Kropf-Gomez, Mr. Rory C. Lang, and Dr. Milton A. Matthews of the Center for Biological Macrofouling Research at the University of Texas at Arlington assisted with experimental determinations of zebra mussel upper thermal limits. Dr. Matthews and Mr. Michael Clarke provided valuable editorial assistance with earlier versions of the manuscript.

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During the conduct of this study, Dr. Theriot was Chief, Aquatic Ecology Branch; Dr. Conrad J. Kirby was Chief, Ecological Research Division; and Dr. John W. Keeley was Director, EL, WES.

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1 Introduction

Thermal treatment is an accepted nonchemical mitigation technology for control of raw water system macrofouling by invertebrates (Jenner and Janssen-Mommen 1992; McMahon 1990; Stock and Del La Parra 1983; Stone and Webster Engineering Corporation 1984; Stone and Webster Environmental Services 1993). For *Dreissena polymorpha*, the zebra mussel, and other macrofouling species, upper lethal thermal (LT) limits have generally been determined as the period of time over which a sample of individuals can tolerate continuous exposure to a specific series of elevated temperatures. The results of such tests are generally expressed as the LT_{50} value, the estimated time required for induction of 50-percent sample mortality, or LT_{100}, the estimated time required for induction of near 100-percent mortality at any one test temperature (Iwanyzki and McCauley 1992; Stock and Del La Parra 1983). This type of temperature tolerance testing involves long-term holding of test individuals at a specific "acclimation" temperature, followed by instantaneous transfer into a series of constantly maintained lethal test temperatures and recording of survival times. The temperature tolerance values resulting from such studies are known as "upper incipient lethal temperatures" or "chronic lethal temperatures" (Stirling 1982) and are expressed as tolerated times of exposure to constant temperatures (i.e., LT_{50} = estimated time of exposure that is lethal to 50 percent of sampled individuals at a specific lethal temperature, and LT_{100} = estimated time of exposure that is lethal to nearly 100 percent [99.9 percent] of sampled individuals).

While the upper incipient lethal temperature approach is useful for comparing the upper thermal limits of different animal species or populations, this value is of less utility for accurate determination of the minimal temperatures required to mitigate infestations of biofouling organisms such as zebra mussels because infested raw water systems are neither able to instantaneously increase operational raw water temperatures to lethal levels nor able to maintain elevated water temperatures for extended periods. Indeed, for many raw water systems, operation above normal water temperatures can reduce efficiency and increase component wear, making treatment of zebra mussels by prolonged maintenance of elevated temperatures economically infeasible. Therefore, a more efficacious thermal treatment may involve increasing raw water temperature until the temperature of instantaneous 100-percent mussel mortality is achieved followed by rapid return to normal operating temperature, greatly reducing the duration of inefficient operation and component wear. Warming

to the instantaneous lethal temperature may also be a particularly applicable means of zebra mussel mitigation in off-line components such as intake embayments heated by steam injection (Kovalak 1993), or in various isolated sections or components of mussel-fouled raw water systems warmed by steam injection or other means (Miller et al. 1992).

Any experimental approach to the determination of upper thermal limits in zebra mussels and other biofouling organisms should mimic actual operating conditions at the facilities applying thermal treatments. This would generally involve an increase in the water temperature of the system through either thermal backwash, recirculation of thermal discharge, steam or hot water injection, or implementation of other water heating devices (submerged heating coils, temporary reduction of flow in specific heat exchangers, etc.). The rate at which operating water temperature is increased may be equally important as the operating water temperature prior to thermal treatment in determining the temperature required to achieve instantaneous 100-percent kill of zebra mussel infestations. Thus, a more appropriate form of thermal tolerance testing for zebra mussels and other biofouling organisms may involve subjecting specimens to a specific heating rate and determining the temperature of instantaneous mortality (i.e., LT_{50} = estimated temperature instantaneously lethal to 50 percent of the sampled individuals and LT_{100} = estimated temperature instantaneously lethal to near 100 percent [99.9 percent] of sampled individuals) without prolonged maintenance at a specific lethal temperature. Thermal tolerance values derived in this manner are called "acute upper lethal temperatures" (AULT). AULT values have generally been determined only for marine intertidal molluscs (Stirling 1982) and have not been measured in biofouling species. In addition, AULT determinations have utilized temperature increase rates of only 1 °C/5 min (1.8 °F/5 min) or 1 °C/10 min (1.8 °F/10 min) (Stirling 1982), which do not realistically reflect the range of heating rates that could be achieved in many raw water systems.

In order to provide baseline data for the further development of a zebra mussel thermal mitigation strategy involving increasing raw water system temperatures to the AULT for instantaneous 100-percent mussel mortality followed by rapid return to normal operating temperature, the effects of temperature increase rates and prior temperature experience (i.e., acclimation temperature) on the AULT values of zebra mussels were assessed. Unlike previous AULT determinations for other mollusc species (Stirling, 1982), zebra mussel AULT values assessed as LT_{50}, LT_{100}, and SM_{100} values (SM_{100} = actual temperature recorded for 100-percent sample mortality) were determined for specimens acclimated to a wide range of temperatures (representing possible raw water intake operating temperatures) and subjected to a wide range of temperature increase rates (representing possible raw water system heating rates). The data allowed development of mathematical models predicting the upper lethal temperatures necessary to be surpassed for 100-percent mitigation of mussel fouling based on a system's prior raw water intake operating temperature and the water heating rate to which mussels were exposed.

2 Materials and Methods

Zebra mussels were collected at a U.S. Army Corps of Engineers navigation lock on the Niagara River in western New York State. Immediately following collection, mussels were shipped overnight in insulated, cooled containers to the Center for Biological Macrofouling Research at the University of Texas at Arlington, where they were maintained in a 200-ℓ (75 gal) refrigerated "Living Stream" holding tank at a constant temperature of 5°C (41 °F) without feeding in dechlorinated City of Arlington tap water until utilized within four months of collection. Zebra mussels have been held in the laboratory in good condition in this manner for periods of over one year.

Groups of mussels were removed from the holding tank and transferred to plastic tanks (22 cm deep x 21 cm wide x 31 cm long) containing 17 ℓ (4.5 gal) of dechlorinated City of Arlington tap water. Tanks were held in refrigerated incubators in which mussels were acclimated to constant temperatures of 10, 15, 20, 25, or 30 °C (50, 59, 68, 77, or 86 °F) (± 0.5 °C or 0.9 °F) for a period of greater than 14 days prior to determination of AULT. Mussels acclimated to 5 °C (41 °F) were drawn directly from the 200-ℓ, 5 °C "Living Stream" holding tank. The acclimation tanks medium was replaced every 7 days for mussels held at 20 and 25 °C and every 3 days for mussels acclimated to 30 °C with water at the temperature of acclimation. There was little, if any, mortality observed in mussels held at any of the acclimation temperatures.

After acclimation to a specific temperature, mussels were divided into subsamples of approximately 10 adult individuals each (overall subsample n range = 7-19, overall shell length range of subsampled individuals = 7.8 to 29.6 mm) for determination of AULT. Subsampled individuals were byssally attached to each other or to the shells of dead mussels. Byssally attached mussels were utilized because removal from the byssus has been demonstrated to reduce the tolerance of zebra mussels to stress, such as that induced by biocide exposure (McMahon, Shipman, and Long 1992). In addition, mussel clusters mimicked conditions in fouling populations where individuals are byssally bound to each other to form dense encrusting mats (Kovalak, Langton, and Smithee 1992; McMahon 1990).

The thermal tolerance of mussels held at each acclimation temperature was determined as the acute upper lethal temperature limit (Stirling 1982).

Subsamples from a group of mussels acclimated to a specific temperature were placed individually into ten 5- by 5-cm, 60-ml glass jars. Jars were covered with 1-mm nylon mesh held in place by a rubber band to prevent mussel escape. Jars were submerged in a 25-cm-deep by 22-cm-wide by 43-cm-long insulated water bath containing 23 ℓ of dechlorinated tap water constantly cooled by a Forma Scientific, Refrigerated Cold Finger (Model 2535). Water in the bath was circulated and initially held at the sampled mussels' acclimation temperature ($\pm$ 0.01 °C) by a Haake D1 Water Bath Temperature Regulator. Rapid water circulation assured uniform temperature throughout the bath. After habituation of subsamples to bath conditions at the temperature of acclimation, bath water temperature was raised by manual adjustment of the temperature regulator to achieve specific heating rates (i.e., 1 °C (1.8 °F) per 5, 10, 15, 20, 30, 45, or 60 min).

Throughout AULT determinations, bath water temperature was continually monitored with a fast-responding micro-thermistor and a Model 43-DT, Yellow Springs Instrument Company Tele-Thermometer. At each tested rate of temperature increase, a subsample of mussels was withdrawn from the bath with each successive 1 °C increase in bath temperature following attainment of a predetermined temperature limit low enough that it did not induce mortality. Removal of 10 separate subsamples at subsequent 1 °C-temperature increases above this initial temperature always resulted in attainment of the temperature of acute 100-percent instantaneous mortality within the 10 °C range of temperature subsamples experienced above the initial lower temperature limit.

After removal from the bath, water in the holding jars was allowed to cool to room temperature (22 to 23 °C). After one hour of recovery, viability of mussels was determined by gentle touching of the tissues of the posterior mantle edge or siphons with the bristles of a fine brush. If this tactile stimulation did not elicit a valve closure response, the mantle edges and siphons were more vigorously probed with the hard, pointed end of the brush handle. If this more vigorous tactile stimulation still did not elicit valve closure, the individual was considered to be dead. This procedure was repeated after the samples had recovered at room temperature for 12 hr. After viability testing, the shell length (SL, the shortest linear distance between the posterior margin of the shell and the anterior tip of the umbos) of each mussel in the subsamples was measured to the nearest 0.1 mm with dial calipers.

3 Results

All mussels which did not display a valve closure response after 1 hr of recovery from exposure to elevated temperatures did not recover after 12 hr at room temperature. Similarly, all mussels displaying a valve closure response after 1 hr of recovery were also capable of valve closure after 12 hr of recovery. Therefore, percent mortality was estimated only from observations of mussel viability after the 1-hr recovery period.

In order to determine if individual mussel size measured as shell length (SL) affected AULT, the ratio of mean SL of all dead individuals to the mean SL of all individuals in a particular subsample (termed "mortality SL ratios") was determined for subsamples in which mortality was greater than 0 percent but less than 100 percent. Mean mortality SL ratios were then computed for those samples drawn from bath temperatures at which mortality was first recorded and for samples taken at the subsequent 1 °C increase in temperature above that at which mortality was first recorded. Means were computed across all tested temperature acclimation groups and heating rates. The 95-percent confidence limits of these means overlapped 1.0 (mean SL mortality ratio at first temperature of recorded mortality = 1.03, 95-percent confidence limits of mean = $\pm$ 0.056; mean SL mortality ratio at second temperature of recorded mortality = O.976, 95-percent confidence limits of mean = $\pm$0.074). One-way Analysis of Variance indicated that these mean ratios were not significantly different from each other ($P < 0.05$). These results indicate that SL did not influence mortality levels within the tested size range (7.8-29.6 mm) or over different test temperatures. Therefore, individual size was not included as a factor in all further analysis of AULT.

Percent subsample mortality values ranging from the highest temperature at which 0-percent subsample survival was recorded to that at which 100-percent subsample mortality was first achieved were subjected to probit analysis (Bliss 1936) to estimate AULT as LT_{50} (i.e., estimated temperature inducing 50-percent sample mortality) and LT_{100} (i.e., estimated temperature inducing 99.9-percent sample mortality). The lowest temperatures at which 100-percent sample mortality was observed (SM_{100}) were also recorded. These values were recorded for all tested combinations of acclimation temperature and heating rates (Table 1).

Table 1
Effect of Temperature Acclimation on the Instantaneous Upper Lethal Temperatures of *Dreissena polymorpha* on Exposure to Different Rates of Temperature Increase

Acclimation Temperature °C	Rate of Temperature Increase min/1 °C	LT_{50} °C	LT_{100} °C	Temperature of 100% Sample Mortality, °C	Sample Size Range
5	5	35.28	37.52	37	8-14
	10	34.67	36.18	36	9-13
	15	34.13	35.07	35	13-15
	20	34.50	35.00	35	8-14
	30	34.01	34.95	35	8-9
	45	33.73	35.26	35	9-12
	60	33.95	35.43	35	8-11
10	5	36.26	38.16	37	8-14
	10	35.44	37.46	37	11-12
	15	35.64	37.13	37	8-14
	20	35.12	36.06	36	9
	30	36.01	36.94	37	9-15
	45	34.17	36.28	36	11-16
	60	34.63	36.11	36	7-10
15	5	36.16	37.10	37	8-9
	10	36.14	38.25	38	8-11
	15	36.5	37.00	37	10-11
	20	35.72	37.25	37	11-14
	30	36.01	36.94	37	10-18
	45	35.01	37.04	37	10-19
	60	35.12	36.06	36	11-14
20	5	38.32	39.26	39	12-15
	10	36.57	39.39	38	8-13
	15	37.60	39.10	39	10-16
	20	36.85	38.27	38	9-12
	30	35.82	37.21	37	10-14

(Continued)

Table 1 (Concluded)

Acclimation Temperature °C	Rate of Temperature Increase min/1 °C	LT_{50} °C	LT_{100} °C	Temperature of 100% Sample Mortality, °C	Sample Size Range
	45	35.62	37.11	37	12-15
	60	35.14	36.08	36	10-13
25	5	39.29	41.52	41	9-16
	10	38.1	39.04	39	10-12
	15	38.09	39.02	39	11-12
	20	37.97	38.90	39	11-12
	30	36.60	38.12	38	10
	45	36.02	36.95	37	10-14
	60	36.04	36.97	37	11-12
30	5	40.07	41.00	41	11-13
	10	39.06	39.99	40	10-11
	15	37.74	40.55	40	10-11
	20	37.08	39.16	39	9-10
	30	37.03	39.15	39	10-11
	45	35.76	37.32	37	9-10
	60	36.1	37.04	37	10-11

Two-way Analysis of Variance indicated that both acclimation temperature ($P < 0.00001$) and rate of temperature increase ($P < 0.00001$) significantly affected all three computed AULT values (i.e., LT_{50}, LT_{100}, or SM_{100}), AULT increasing with increased acclimation temperature over 5 to 30 °C (41 to 86 °F) and decreasing with decreased heating rates over 1 °C/5 min to 1 °C/60 min (1.8 °F/5 min to 1.8 °F/60 min) (Table 1).

Least Squares Multiple Linear Regression Analysis indicated that all three measures of AULT were positively correlated with temperature of acclimation and negatively exponentially related to heating rate expressed as minutes required to raise temperature 1 °C ($P < 0.0001$), allowing the relationship between AULT and both acclimation temperature and heating rate to be expressed as multiple regression equations in which the natural logarithm of AULT (as LT_{50}, LT_{100}, or SM_{100}) was the dependent variable and acclimation temperature and the natural logarithm of heating rate were the independent variables (Table 2). The r values for all three regressions ranged from 0.91-0.92 (Table 2), indicating that the effects of acclimation temperature and temperature increase rate accounted for over 90 percent of the observed variation in AULT values.

Table 2
Multiple Linear Regressions Predicting Acute Upper Lethal Temperature (AULT) as LT_{50}, LT_{100}, or SM_{100} Values (°C) in Zebra Mussels (*Dreissena polymorpha*) Relative to Acclimation Temperature (°C) and Rate of Temperature Increase (Min/1°C)

Basic Regression Equation							
AULT, °C	a	b	c	n	r	F	P
LT_{50}	3.603	-0.026	0.0036	42	0.92	b = 162.1 c = 75.0	<0.0001 <0.0001
LT_{100}	3.647	-0.029	0.0037	42	0.91	b = 133.6 c = 72.5	<0.0001 <0.0001
SM_{100}	3.629	-0.025	0.0038	42	0.92	b = 159.0 c = 62.9	<0.0001 <0.0001

The relationship between AULT as LT_{50}, LT_{100}, or SM_{100} and acclimation temperature and heating rate predicted by the regression equations in Table 2 is illustrated in Figures 1, 2, and 3. As depicted in these figures, the LT_{50} of adult zebra mussels increases roughly 0.13 °C for every 1 °C increase in acclimation temperature (0.072 °F/1 °F acclimation temperature), while LT_{100} and SM_{100} both increase approximately 0.14 °C per 1 °C increase in acclimation temperature (0.078 °F/1 °F) (Figures 2 and 3). Thus, if zebra mussels experiencing a raw water system operating temperature of 0 °C (32 °F) were subjected to a temperature increase of 1 °C or 1.8 °F/5 min, a final temperature of 35.2 °C (95 °F) would have to be achieved to induce 50-percent mortality in fouling populations (LT_{50}, Figure 1, Table 2) and a final temperature of 36.6 °C (97.9 °F) would be required to achieve 100-percent mortality (LT_{100}, Figure 2, Table 2). The corresponding predicted temperature required to achieve 100-percent mortality based on direct laboratory observations (i.e., SM_{100} values) would be 36.2 °C (97.1 °F) (Figure 3, Table 2). At the same operating temperature of 0 °C, but at a heating rate of 1 °C or 1.8 °C/60 min, required final AULT values would decline to 33.0 °C (91.4 °F) for LT_{50}, 34.1 °C (93.3 °F) for LT_{100}, and 34.0 °C (93.2 °F) for SM_{100}. If operating temperature was 15 °C (59 °F) and heating rate, 1 °C/5 min, these final AULT values would be 37 °C (98.9 °F) for LT_{50}, 38.7 °C (101.7 °F) for LT_{100}, and 38.3 °C (101.0 °F) for SM_{100}, but if heating rate was 1 °C/60 min, they would decline to 34.8 °C (94.7 °F) for LT_{50}, 36.0 °C (96.8 °F) for LT_{100}, and 36.0 °C (96.8 °F) for SM_{100}. If the operating temperature was 30 °C (86 °F), and heating rate was 1 °C/5 min, these values would be 39.2 °C (102.6 °F) for LT_{50}, 40.9 °C (105.6 °F) for LT_{100}, and 40.6 °C (105.0 °F) for SM_{100}, but if heating rate was 1 °C/60 min they would decline to 36.8 °C (98.2 °F) for LT_{50}, 38.1 °C (100.5 °F) for LT_{100}, and 38.1 °C (100.6 °F) for SM_{100} (Table 2, Figures 1, 2 and 3).

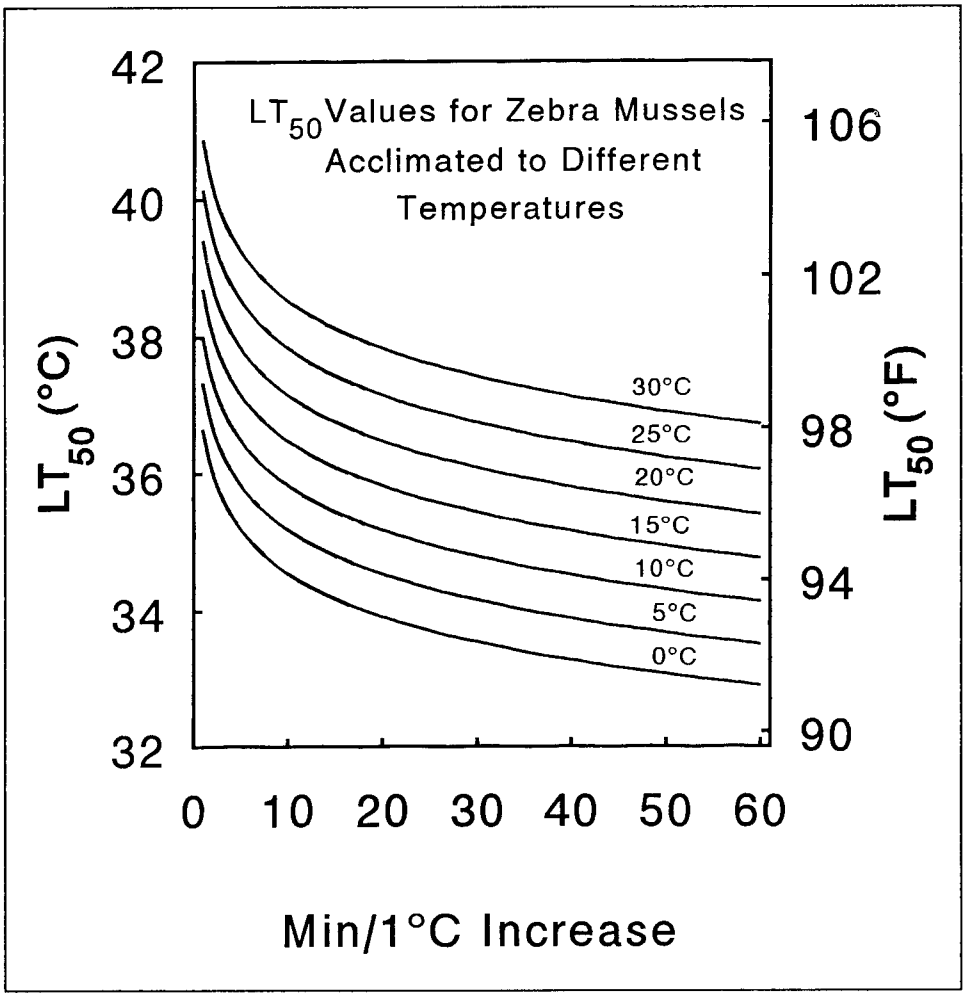

Figure 1. Acute upper lethal temperature in zebra mussels (*Dreissena polymorpha*) measured as LT_{50}, the estimated temperature of instantaneous 50-percent sample mortality (indicated on the left vertical axis in °C and on the right vertical axis in °F), relative to rate of water warming in minutes per 1 °C (horizontal axis)

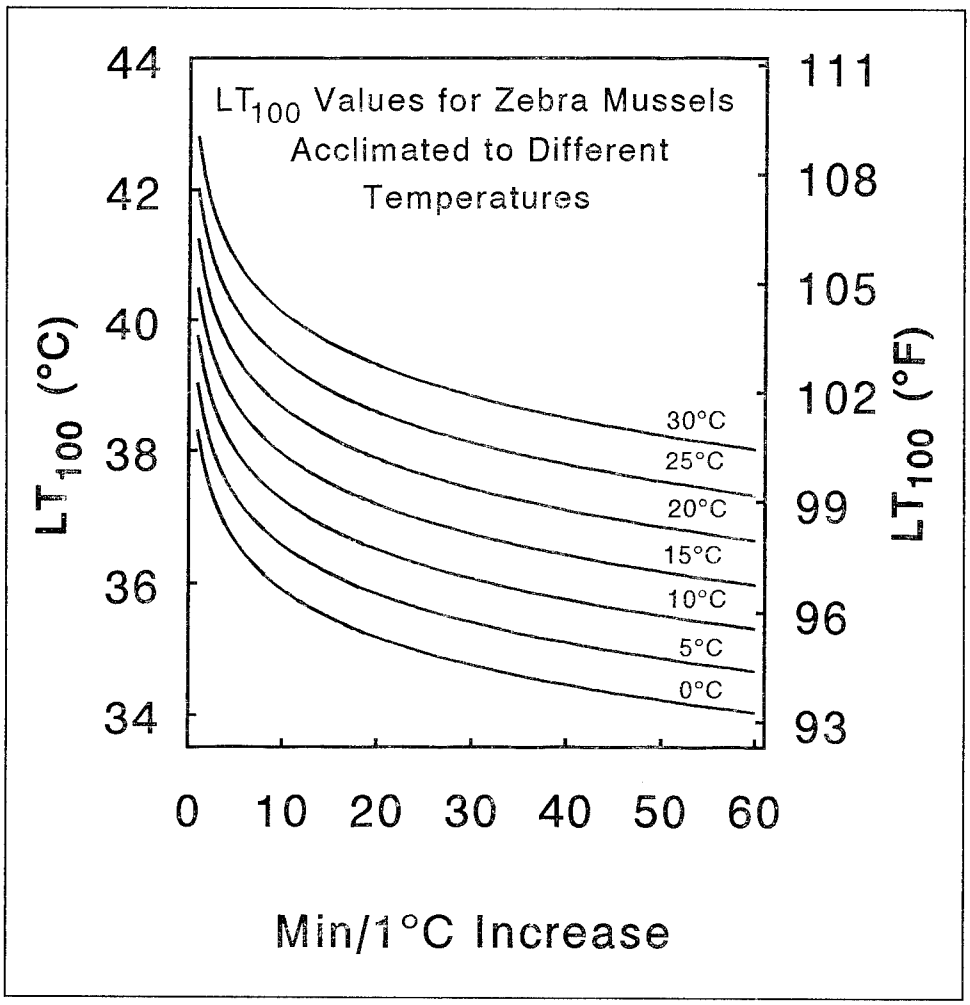

Figure 2. Acute upper lethal temperature in zebra mussels (*Dreissena polymorpha*) measured as LT_{100}, the estimated temperature of instantaneous 100-percent sample mortality (indicated on the left vertical axis in °C and on the right vertical axis in °F), relative to rate of water warming in minutes per 1 °C (horizontal axis)

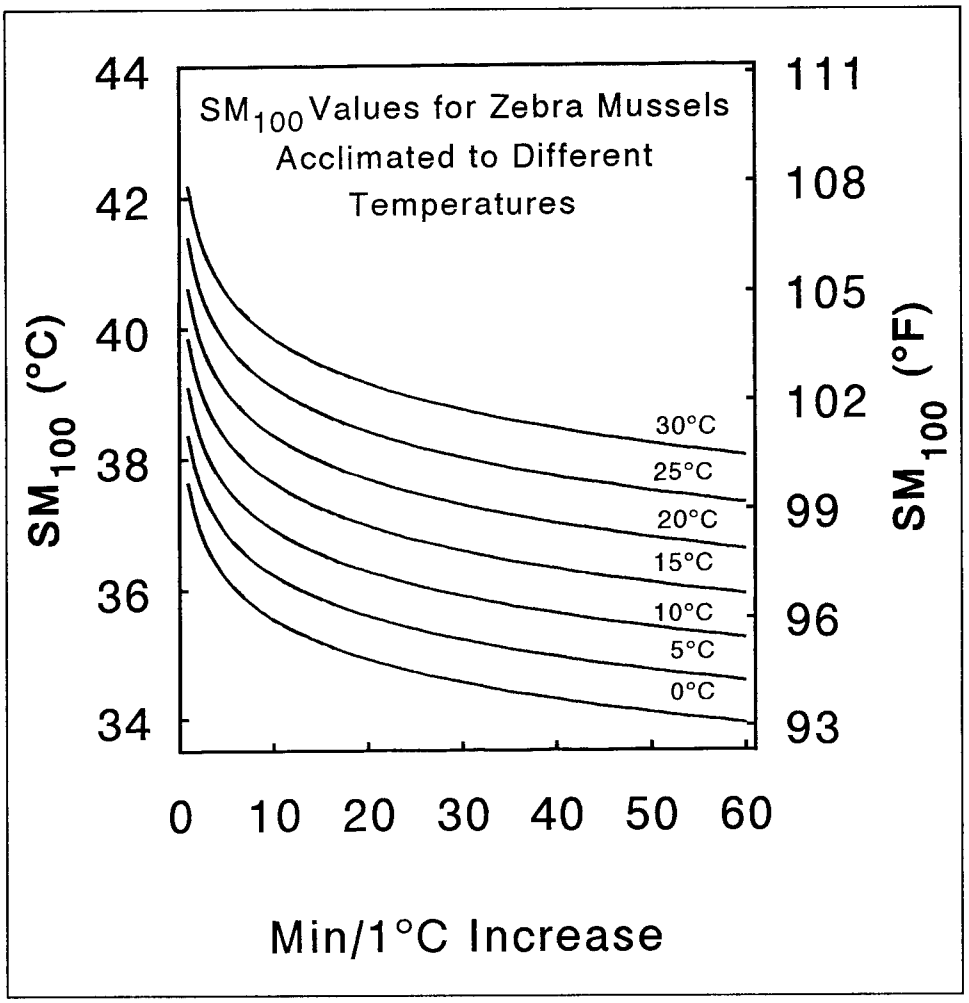

Figure 3. The relation of acute upper lethal temperature in zebra mussels (*Dreissena polymorpha*) measured as SM_{100}, the estimated actual lowest temperature at which instantaneous 100-percent sample mortality was first observed (indicated on the left vertical axis in °C and on the right vertical axis in °F), relative to rate of water warming in minutes per 1 °C (horizontal axis)

4 Discussion

The thermal tolerance values of *D. polymorpha* presented in this report as acute upper lethal temperatures under different heating rates are not strictly comparable to thermal tolerance values previously published for this species, as these have all been determined as upper incipient or chronic temperature tolerance times on exposure to specific lethal temperatures. Jenner and Janssen-Mommen (1992) have shown that zebra mussels acclimated to prevailing natural water temperatures in the Netherlands have an incipient upper temperature tolerance of less than 10 min at 36 °C (96.8 °C), which increases to 1.5 hr at 33 °C (91.4 °F). Mean tolerance times of North American zebra mussels from Lakes Erie and St. Clair when exposed to 30 °C varied between 4.74 days when specimens where acclimated to 2.5 °C (36.5 °F) and 3.96 days when acclimated to 25 °C (77 °F) (Iwanyzki and McCauley 1992). At 33 °C (91.4 °F), these values declined to 0.22 and 17.5 hr in mussels acclimated to 2.5 and 25 °C, respectively, and further declined to 0.17 and 0.65 hr, respectively, when 2.5 and 25 °C-acclimated mussels were exposed to 36 °C (Iwanyzki and McCauley 1992). The mean incipient temperature tolerance times recorded by Iwanyzki and McCauley (1992) for North American zebra mussels were higher than those reported for 100-percent mortality in zebra mussels from northern Europe (Jenner and Janssen-Mommen 1992), even though Iwanyzki and McCauley (1992) reported mean values which underestimate the duration of temperature exposure required to achieve 100-percent mortality. However, their values are similar to those quoted from other, unpublished sources (Iwanyzki and McCauley 1992) for North American zebra mussel populations from Lake Erie and Rybinskoye Vodokhranifishche Reservoir in northwestern Russia.

The AULT values presented in this report are also indicative of a higher level of thermal tolerance in North American zebra mussels than reported for this species in northern Europe (Jenner and Janssen-Monnen 1992). At heating rates of 1 °C/60 min, approximating a long-term lethal temperature exposure, AULT as LT_{100} in zebra mussels from the Niagara River ranged from 34.7 °C (94.5 °F) when specimens were acclimated to 5 °C (41 °F) to 38.8 °C (101.9 °F) when specimens were acclimated to 30 °C (86 °F). The zebra mussel upper lethal temperature limits determined in this study also appear elevated relative to other values reported as incipient upper lethal temperatures for North American zebra mussel populations (Iwanyzki and McCauley 1992 and unpublished reports cited therein). Iwanyzki and

McCauley (1992) estimated that 30 °C (86 °F) was the minimum incipient upper lethal temperature for zebra mussels taken from Lakes St. Clair and Erie with mean time to death at this temperature ranging from 2.6 to 4.7 days in mussels acclimated to a temperature range of 2.5 ° to 25.0 °C (36.5 to 77 °F). In contrast, mussels were maintained during the course of this study for greater than 67 days at 30 °C without extensive mortality, suggesting that the minimal incipient upper lethal temperature of North American zebra mussels is greater than 30 °C.

The thermal tolerance of *D. polymorpha*, whether measured as incipient upper lethal temperature limit (Iwanyzki and McCauley 1992; Jenner and Jassen-Mommen 1992) or acute upper lethal temperature (this study), is lower than that of other common North American macrofouling bivalve species. At 32.2 °C (90 °F), 95-percent mortality was induced in specimens of the marine, macrofouling, blue mussel, *Mytilus edulis* L., within 23 hr, with tolerance time decreasing to 0.23 hr at 40.5 °C (105 °F) (Stock and Del La Parra 1983). In another study, maintenance of blue mussels at 35 °C (95 °F) for 1 hr induced 56-percent sample mortality, and at 40 °C (104 °F), 100-percent sample mortality was induced within 0.33 hr (Johnson et al. 1983). The short-term upper thermal limit of the Atlantic or American oyster, *Crassostrea virginica* Gmelin, is 48.5 °C (119.3 °F) (Sellers and Stanley 1989), a value much higher than that of *D. polymorpha*. The elevated temperature tolerance of the American oyster is likely to be an adaptation to its shallow-water, thermally unstable, estuarine habitats. The freshwater, macrofouling Asian clam, *Corbicula fluminea* (Müller) is also considerably more thermally tolerant than *D. polymorpha*. The instantaneous upper lethal temperature of Asian clams is approximately 44 °C (111.2 °F) in individuals acclimated to 32 °C (89.6 °F), with minimal tolerated temperature being 30 °C (86 °F) in 5 °C (41 °F)-acclimated clams which survive exposure to this temperature for less than 4-7 hr (Mattice 1979). In *C. fluminea*, 36 °C is the minimum long-term incipient upper lethal temperature (McMahon and Williams 1986). The elevated upper thermal limits of *C. fluminea* relative to *D. polymorpha* reflects its endemic distribution in tropical and subtropical southeast Asia (Morton 1979).

The reduced thermal tolerance of *D. polymorpha* relative to other North American biofouling species makes it more susceptible to thermal mitigation. However, as the data presented in this paper and elsewhere (Iwanyzki and McCauley 1992) clearly demonstrate, the zebra mussel is capable of extensive temperature acclimation of its acute upper lethal temperature limits, its tolerated temperature at any heating rate increasing approximately 0.13 to 0.14 °C (0.23 to 0.25 °F) for each 1 °C (1.8 °F) increase in acclimation temperature (i.e., raw water intake temperature) (Table 2 and Figures 1, 2 and 3). Thus, a raw water system will need to be heated to higher temperatures to achieve 100-percent mitigation of mussel infestations during summer months when source water temperatures are above 20 °C (>77 °F) than in winter months when source water temperatures fall below 5 °C (<41 °F) (Figures 2 and 3).

The data presented here also demonstrate that the rate of water heating, when expressed as °C/min, has a profound exponentially positive effect on the acute upper lethal temperature of *D. polymorpha* such that increased heating rate increases the temperature required to induce 100-percent mortality (Table 2, Figures 1, 2 and 3). Based on the equations in Table 2, the increase in AULT as either LT_{50}, LT_{100}, or SM_{100} with increased heating rate is most pronounced at heating rates of less than 1 °C/10 min (1.8 °F/10 min). Thus, regulation of raw water system heating rates at longer than 1 °C/10 min during thermal treatment of mussel infestations could reduce the temperature required to achieve 100-percent mitigation by 1 to 2 °C (1.8 to 3.6 °F).

5 Conclusions

The data presented strongly suggest that heating of raw water systems to the acute upper lethal temperature of zebra mussels followed by rapid return to normal operating temperatures is a promising thermal mitigation technology for zebra mussel macrofouling, particularly as it has the lowest level of thermal tolerance among common North American macrofouling bivalve species. Based on the model equations relating intake water temperature and system heating rate to temperature of 100-percent instantaneous mortality, the maximum temperature required for 100-percent mussel kill would be 43 °C (109 °F) if mussels were maximally acclimated to 30 °C (86 °F) and subjected to a rapid heating rate of 1 °C/min (1.8 °F/min). The acute temperature of 100-percent instantaneous mortality declines with reduced intake water temperatures and system heating rates such that temperatures of less than 38 °C (95 °F) could induce 100-percent mortality if acclimation temperature was below 20 °C (77 °F) and the system heating rate was slower than 1 °C/20 min (1.8 °F/ 20 min). A thermal mitigation strategy based on warming system water temperature above the acute upper lethal temperature of zebra mussels eliminates the necessity for prolonged operation at elevated temperatures, which in turn increases efficiency of operation and minimizes thermally induced component wear (Stock and Del La Parra 1983).

This research and that of others (Iwanyzki and McCauley 1992) indicates that the upper thermal tolerance levels of North American zebra mussels are elevated by 2 to 3 °C (3.6 to 5.4 °F) over those recorded for this species in Northern Europe (Jenner and Janssen-Mommen 1992). While available data are sparse and plagued by incongruent protocols for measurement of thermal tolerance, the results discussed above do appear to suggest that North American zebra mussels may have originated from a population in the southern portion of this species' present European range, where elevated ambient water temperatures may have selected for a more thermally tolerant physiological race than exists in the cooler freshwaters of northern Europe. Further evidence of a southern European origin for zebra mussels introduced into the Great Lakes is the concurrent introduction of a second, as yet unidentified, species of *Dreissena* in North America (the so-called "Quagga Mussel") (Conn 1992). *Dreissena polymorpha* is the only dreissenid species found in the freshwaters of northern Europe (Mackie et al. 1989). In contrast, several species of freshwater dreissenid mussels including *D. polymorpha* occur in the Black, Aral, and Caspian Seas and their tributaries (Zhadin 1952; Marelli

1991; MacNeil 1991), making this region the likely source of the two dreissenid species introduced to North America. This region is at the most extreme southeastern and likely warmest portion of the distribution range for the zebra mussels in Europe. Therefore, any mussels introduced to North America from this region could have been drawn from a genetically, thermally tolerant population. If this proves to be the case, zebra mussels are likely to extend much further south into the freshwater drainage systems of the United States than has been previously estimated from available information on the temperature tolerance of northern European zebra mussel populations (McMahon 1990; Strayer 1991).

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13. ABSTRACT *(Maximum 200 words)*

For *Dreissena polymorpha* and other macrofouling species, upper lethal thermal limits are generally expressed as tolerance times of specific lethal temperatures. In some raw water systems, reduced efficiency and increased component wear make prolonged maintenance of elevated temperatures for thermal treatment of biological macrofouling infeasible. A more efficacious thermal treatment could involve increase in water temperature until instantaneous 100-percent mortality is achieved followed by rapid return to normal operating temperatures. To provide baseline data for this strategy, the effects of rate of temperature increase and prior temperature experience (i.e., acclimation temperatures) on acute upper lethal temperature (AULT) of zebra mussels were assessed. Mussels were acclimated >14 days to 5, 10, 15, 20, 25, and 30 °C. Their AULTs were determined as LT_{50}, LT_{100} (i.e., estimated temperatures of 50-percent and near 100-percent sample mortality), and SM_{100} values (i.e., lowest actual temperature of 100-percent sample mortality). AULT values were assessed for mussels exposed to heating rates of 1 °C per 5, 10, 15, 20, 30, 45, and 60 min. Multiple linear regression analysis indicated that natural logarithmic transformations of LT_{50}, LT_{100}, or SM_{100} were positively correlated with acclimation temperature ($n = 35$, $P < 0.00001$) and

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negatively correlated with the natural logarithmic transformation of heating rate expressed as minutes required to increase temperature 1 °C (P = 0.00001). Resultant predictive equations were:

$$\ln LT_{50} = 3.603 - 0.026 (\ln \text{min}/1\ °C) + 0.0036 (°C \text{ Acclimation Temperature})$$
$$\ln LT_{100} = 3.647 - 0.029 (\ln \text{min}/1\ °C) + 0.0037 (°C \text{ Acclimation Temperature})$$
$$\ln SM_{100} = 3.629 - 0.025 (\ln \text{min}/1\ °C) + 0.0038 (°C \text{ Acclimation Temperature})$$

Based on these regressions, mussels at an operating temperature of 5 °C would experience 50-percent mortality on reaching a temperature of 35.8 °C (96.4 °F) and 100-percent mortality at 37.3 °C (99.1 °F) if temperature was raised 1 °C/5 min. If temperature was raised 1 °C/60 min, these values decline to 33.6 °C (92.4 °F) and 34.7 °C (94.4 °F), respectively. At an operating temperature of 25 °C and an increase of 1 °C/5 min, mussels would experience 50-percent mortality at 38.5 °C (101.3 °F) and 100-percent mortality at 40.2 °C (104.5 °F), while at 1 °C/60 min these values decline to 36.1 °C (97.0 °F) and 37.4 °C (99.3 °F), respectively. Northern European data suggest that the long-term LT_{100} of zebra mussels is $\approx$33 °C (91.4 °F). The data reported herein indicate that upper thermal limits of North American zebra mussels are likely to be higher than this value, suggesting that North American populations may have been founded by individuals introduced from the southern portion of the mussel's European range.